

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Hyman et al.	)	Examiner:
			)	E. M. Mercader
Serial No.	:	10/001,643	)	
			)	Art Unit:
Cnfrm. No.	:	2817	)	3737
			)	
Filed	:	October 31, 2001	)	
			)	
For	:	IN VIVO MULTIPHOTON DIAGNOSTIC	)	
		DETECTION AND IMAGING OF A	)	
		NEURODEGENERATIVE DISEASE	)	

DECLARATION OF WATT W. WEBB UNDER 37 C.F.R. § 1.132

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Sir:

I, WATT W. WEBB, pursuant to 37 C.F.R. § 1.132, declare:

1. I received a B.S. degree in Business and Engineering Administration from Massachusetts Institute of Technology, Cambridge, Massachusetts in 1947, and an Sc.D. degree in Metallurgy, with a minor in Physics and Mathematics, from Massachusetts Institute of Technology, Cambridge, Massachusetts in 1955.

2. I am currently Professor of Applied Physics, S.B. Eckert Professor in Engineering, and Director of the Developmental Resource for Biophysical Imaging and Opto-Electronics of the School of Applied and Engineering Physics at Cornell University, Ithaca, New York.

3. As indicated in my attached *Curriculum Vitae* (**Exhibit A**), list of publications (**Exhibit B**), and list of published abstracts (**Exhibit C**), I have authored or co-authored over 270 peer-reviewed professional publications and over 290 published abstracts in the fields of biological physics and condensed matter. Since 1992, I have given over 160 invited lectures in these same technical fields (**Exhibit D**).

4. I am an elected Fellow of the American Physical Society, the Biophysical Society, the American Association for the Advancement of Science, and a Founding Fellow of the American Institute of Biological and Medical Engineers. I am an elected Member of the National Academy of Engineering, the National Academy of Science, and the American Academy of Arts and Science.

5. A major focus of my research has been in the area of multiphoton excitation and, as a result of that research and the corresponding publications and lectures, I am regarded as an expert in that field.

6. I am a co-inventor of the above-identified patent application.

7. It is my understanding that the claims of my above patent application are rejected under 35 U.S.C. § 103 for obviousness over U.S. Patent No. 6,329,531 to Turner et al. ("Turner") in view of U.S. Patent Application Publication No. 2003/0236458 to Hochman ("Hochman").

8. Turner relates to *in vivo* and *in vitro* diagnosis of neurodegenerative diseases such as Alzheimer's Disease by means of near infra-red radiation. According to the *in vivo* methods of Turner, one or more dye compounds are fed to the tissue being diagnosed and light from the near-infrared spectral region is irradiated. The non-absorbed, scattered light and/or scattered fluorescence radiation emitted by the dye is recorded simultaneously/individually. Preferred methods are where the tissue irradiates over a large surface, and the fluorescence radiation that is resolved locally is visualized by imaging with a CCD camera or the tissue areas that are to be imaged are rastered with a fiber optic light guide and the signals that are received are converted numerically into a synthetic image. Fluorescence can also be evaluated spectrally and/or by phase selection, as well as in a steady-state manner and/or in a time-resolved manner.

9. Hochman teaches methods for optically detecting physiological properties in an area of interest by detecting changes in the intrinsic or extrinsic optical properties of tissue. This involves optically detecting blood flow changes, blood characteristics, and blood vessel abnormalities, as well as determining the presence and location of abnormal or pathological tissue for identifying and mapping the margins of abnormal tissue. According to Hochman, these methods may be used to identify physiological conditions associated with and to evaluate diagnosis of Alzheimer's Disease and other neurodegenerative disorders. Optical detection may involve invasive or semi-

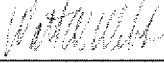
invasive systems and may be continuous or non-continuous (*i.e.*, pulsed). Data sets from patients can be compared to standard or control data representative of optical properties indicative of various disease states or conditions. Longer wavelengths (e.g., approximately 800 nm) can be employed to analyze deeper areas of tissue.

10. Turner and Hochman disclose the use of a class of colorant signal molecules. To the extent these references discuss how they are used, their achievement of fluorescent excitation does not result in a non-linear process like two-photon or multiphoton excitation. The dyes utilized by Turner and Hochman absorb low-energy infrared radiation photons to excite the dye molecules to the low energies corresponding to the infrared photons. In contrast, two-photon or multiphoton excitation absorbs two or more infrared photons virtually simultaneously to excite a molecule to an energy level corresponding to the sum of the energies of the two or more infrared photons. These energy levels can then be high enough to be released by emission of visible or even ultraviolet fluorescence. Since the procedures used by Turner and Hochman do not achieve such a high energy of excitation, it is apparent that they do not carry out simultaneous multiphoton excitation, in accordance with my present invention.

11. Thus, neither Turner or Hochman teach or suggest a method involving activating brain tissue of a mammal by application of radiation through an opening or a thinned portion of the mammal's skull to promote simultaneous multiphoton excitation, where the radiation is pulsed at a pulse width between about  $10^{-9}$  to  $10^{-15}$  second.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: \_\_\_\_\_

  
Digitally signed by Watt W. Webb  
Date: 2008.12.19 11:29:14 -05'00'  
\_\_\_\_\_  
**Watt W. Webb, Sc.D.**



# CURRICULUM VITAE

## WATT W. WEBB

### PERSONAL DATA

Born: Kansas City, MO, August 27, 1927  
 Elementary Education: Silver City, NM; Kansas City, MO  
 Marriage: Page Chapman, November 23, 1950  
 Issue: Watt W. Webb III (1952); Bucknell C. Webb (1957); Spahr C. Webb (1957)  
 Home Address: 9 Parkway Place, Ithaca, New York 14850 (607-257-7592)  
 Office Address: Professor of Applied Physics, S.B. Eckert Professor in Engineering  
 School of Applied and Engineering Physics  
 223 Clark Hall  
 Cornell University  
 Ithaca, New York 14853-2501  
 Office phone: 607-255-3331  
 Office fax: 607-255-7658  
 Web Sites: <http://www.aep.cornell.edu/FFR/Faculty/Webb.html>  
<http://www.aep.cornell.edu/drbio/drbio.html>

### EDUCATION

B.S., 1947, Business and Engineering Administration, Massachusetts Institute of Technology  
 Sc. D., 1955, Metallurgy, minor Physics and Mathematics, Massachusetts Institute of Technology,  
 Thesis: "Oxidation Studies in Metal-Carbon Systems" with Carl Wagner and J.T. Norton

### PROFESSIONAL POSITIONS

1947-1952	Union Carbide Research Laboratories, Research Engineer
1955-1961	Union Carbide Metals Company, Research Scientist (1955-59); Coordinator of Fundamental Research (1959-60); Assistant Director of Research (1960-61)
1961-1965	Associate Professor of Engineering Physics, Cornell University
1965-	Professor of Applied Physics, Cornell University
1965-	S.B. Eckert Professor in Engineering
1965-	Director, School of Applied and Engineering Physics, Cornell University
1983-1988	Director, Developmental Resource for Biophysical Imaging and Opto-electronics
1988-	Faculty of Biological Sciences, Cornell University
1988-	Scholar in Residence, NIH Fogarty International Center for Advanced Study
1989, 1990	Director, Biophysics Program, Cornell University
1989-1992	

### HONORS/AWARDS (Cumulative)

1954	MIT Overseas Fellowship
1953-1955	Allegheny Ludlum Fellowship
1974-1975	Guggenheim Fellowship
1975-	Fellow American Physical Society
1989-	Fellow American Association for the Advancement of Science
1991	Biological Physics Prize of the American Physical Society
1992-	Founding Fellow American Institute for Medical and Biological Engineering
1993-	National Academy of Engineering, elected member
1995-	National Academy of Science, elected member
1997-	American Academy of Arts and Sciences, elected member
1997	Ernst Abbe Lecture Award, Biophysical Society and Royal Microscope Society
1999	Michelson-Morley Award of Case Western Reserve University
1999-	Fellow of the Biophysical Society
1999	Jablonski Award of the Biophysical Society
2000	Rank Prize in Opto-electronics - International
2001	Wenner-Gren Distinguished Lectureship - Sweden
2002	Biophysical Society National Lecturer

**PUBLICATIONS 276**

Over 265 papers in condensed matter and biological physics and 13 U.S. patents plus foreign patents.  
 Invited Lectures – typically 10-15 per year last 5 years  
 Published Abstracts – averaging >13 per year last 5 years

**PROFESSIONAL ACTIVITIES (RECENT AND CURRENT)**

1961-	Consultant in applied physics for industry and government
1975-1991	Associate Editor for Biological Physics, Physical Review Letters
1986-1991	Executive Committee, Vice Chairman, Chairmen and Past Chairman, Division of Biological Physics, American Physical Society.
1984-1997	Council, American Physical Society
1984-1986	Co-chairman, Panel of Scientific Interfaces and Technological Applications of Physics of NAS/NRC Physics Survey
1981	Co-chairman, Interactional Workshop on the Biological Applications of Photobleaching Techniques
1982	Visiting Scholar, Science, Technology and Society Program, Cornell University
Current	Reviewer: Science, Nature, Biophysical Journal, Biochemistry, Physical Review Letters, Physical Review, Journal of Cell Biology, PNAS, Reviews of Modern Physics, Journal of Microscopy, Applied Optics Letters, etc.
Current	Referee: NSF, NIH proposals and site visits, etc.
Current	Memberships: American Physical Society (Fellow); Biophysical Society (Fellow); Society for General Physiologists; American Society of Cell Biology; American Association for the Advancement of Science (Fellow); Optical Society of America, Society for Neuroscience.

**COMMITTEES**

1958-1961	NAS/NRC Committee on Perspectives in Materials Research, Panel on Growth, Structure and Morphology of Crystals
1955-	Advisory panel of MAB, NRC, NSF, NIH, at various times
1963-1964	NAS/NRC Ad Hoc Committee on the Interface Problem in Fibrous Composites
1964-1966	Metallurgical Society Publications Committee
1967-1968	Electrochemical Society Division Executive Committee
1969-1971	Metallurgical Society Committee on the Chemistry and Physics of Metals
1970-1972	Technical Committee ISCYRA
1973-1975	IEEE Magnetics Committee
1974-1977	NSF Materials Science Advisory Committee
1973-1981	Chairman, Cornell Biophysics Advisory Committee
1978-1979	Editorial Board, The Physical Review
1980-1985	Publications Committee, Biophysical Journal
1981-1985	Board of Contributors, Comments on Biophysics
1983-1986	Council and Executive Committee, Biophysical Society
1990-1995	Advisory Committee "Physics Today"
1993-1998	Investment Committee, American Physical Society
1999	Cornell Research Foundation, Executive Committee and Board of Directors (1988-); Chairman, Long Range Planning Committee (1985-88 and 1993-95), Technology Transfer Committee (1986-88)
1998-	Roswell Park Cancer Institute, Buffalo, NY, Science Advisory Board
1998-	Steele Laboratory, Harvard University and Massachusetts General Hospital, Science Advisory Board
1998-	Laser Biomedical Research Center at MIT, Science Advisory Board
1999-	Wellman Laboratory of Photomedicine SAB, Harvard Medical School and Massachusetts General Hospital, Science Advisory Board
2000-	Life Sciences Advisory Council, Cornell University



# PUBLICATIONS OF WATT W. WEBB (Cumulative) as of November 22, 2002

## Number

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- 273 Heikal, A. A. and W. W. Webb, "Multiphoton fluorescence microscopy in biology," in Proceedings of SPIE Annual Meeting 2002, Nonlinear Spectroscopy, Ed(s) D. L. Andrews, SPIE, Bellingham, WA, 4812, pp. 01-, in press 2002
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- 271 Hess, S. T. and W. W. Webb, "Focal Volume Optics and Experimental Artifacts in Confocal Fluorescence Correlation Spectroscopy," *Biophys J.* 83(4), 2300-2317, 2002
- 270 Thompson, R.E., D.R. Larson and W.W. Webb, "Precise Nanometer Localization Analysis for Individual Fluorescent Probes," *Biophys J.* 82(5), 2775-2783, 2002
- 269 Heikal, A.A., S.T. Hess, E.D. Sheets and W.W. Webb, "Mutation-photophysics relationship in intrinsically fluorescent proteins," in Femtochemistry and Femtobiology: ultrafast dynamics in molecular science, Ed(s) A. Douhal and J. Santamaria, World Scientific Publishing Co., London, 2002
- 268 Foquet, M., J. Korlach, W.R. Zipfel, W.W. Webb and H.G. Craighead, "DNA fragment sizing by single molecule detection in submicrometer-sized closed fluidic channels," *Analytical Chemistry* 74(6), 1415-1422, 2002
- 267 Huang, S., A.A. Heikal and W.W. Webb, "Two-Photon Fluorescence Spectroscopy and Microscopy of NAD(P)H and Flavoprotein," *Biophys. J.* 82(5), 2811-2825, 2002
- 266 Hess, S.T., S. Huang, A.A. Heikal and W.W. Webb, "Biological and Chemical Applications of Fluorescence Correlation Spectroscopy: A Review," *Biochemistry* 41(3), 697-705, 2002
- 265 Heikal, A.A., S.T. Hess and W.W. Webb, "Multiphoton molecular spectroscopy and excited state dynamics of enhanced green fluorescent protein (EGFP): acid-base specificity," *Chemical Physics* 274, 37-55, 2001
- 264 Williams, R.M., W.R. Zipfel and W.W. Webb, "Multiphoton microscopy in biological research," *Current Opinion in Chemical Biology* 5, 603-608, 2001



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- 261 Christie, R.H., B.J. Bacskai, W.R. Zipfel, R.M. Williams, S.T. Kajdasz, W.W. Webb, and B.T. Hyman, "Growth arrest of individual senile plaques in a model of Alzheimer's disease observed by *in vivo* multiphoton microscopy," *J. Neuroscience* 21 (3), 858-864, 2001.
- 260 Heikal, A.A., S.T. Hess, G.S. Baird, R.Y. Tsien, and W.W. Webb, "Molecular Spectroscopy and Dynamics of Intrinsically Fluorescent Proteins: Coral Red (DsRed) and Yellow (Citrine)," *PNAS* 97 (22), 11996-12001, 2000; correction in *PNAS* 97 (26), 14831-14831, 2000.
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- 232 Köhler, R. H., W. R. Zipfel, W.W. Webb, and M. R. Hanson, "The Green Fluorescent Protein as a Marker to Visualize Plant Mitochondria *In Vivo*," *The Plant Journal* **11** (3), 613-621, 1997.
- 231 Xu, C., J. B. Shear, and W.W. Webb, "Hyper-Rayleigh and Hyper-Raman Scattering Background of Liquid Water in Two-Photon Excited Fluorescence Detection," *Analytical Chemistry* **69** (7), 1285-1287, 1997.
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- 229 Xu, C., W.R. Zipfel, J.B. Shear, R.M. Williams, and W.W. Webb, "Multiphoton Fluorescence Excitation: New Spectral Windows for Biological Nonlinear Microscopy," *PNAS* 93, 10763-10768, 1996.
- 228 Guild, J.B., C. Xu, and W.W. Webb, "Measurement of Group Delay Dispersion of High Numerical Aperture Objective Lenses Using Two-Photon Excited Fluorescence," *Applied Optics* 36 (1), 397-401, 1997.
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- 224 Mertz, J., C. Xu, and W.W. Webb, "Single-Molecule Detection by Two-Photon Excited Fluorescence," *Optics Lett.* 20 (24), 2532-2534, 1995.
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- 220 Xu, C., J. Guild, W.W. Webb, and Winfred Denk, "Determination of Absolute Two-Photon Excitation Cross-Sections by *In Situ* Second-Order Autocorrelation," *Optics Lett.* 20 (23), 2372-2374, 1995.
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"Imaging of Total Intracellular Calcium and Calcium Influx and Efflux in Individual  
Resting and Stimulated Tumor Mast Cells using Ion Microscopy," *J. of Biol.*  
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Biological Confocal Microscopy, ed. by J. Pawley, 39-53, 1995.
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Electroosmosis Elicits Cytosolic Calcium Response in Tumor Mast Cells," *J. Cell*  
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Laser Scanning Microscopy," Handbook of Biological Confocal Microscopy,  
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an Optical Trap," *Rev. Sci. Instrum.* 65 (9), 2762-2768, 1994.
- 211 Thomas, J.L., D. Holowka, B. Baird, and W.W. Webb, "Large-Scale Co-  
Aggregation of Fluorescent Lipid Probes with Cell Surface Proteins," *J. Cell Bio.*  
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Photon Fluorescence Excitation in Laser Scanning Microscopy Images Calcium  
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Microscopy and Microphotochemistry," *FASEB Journal* 8 (11), 804-813, 1994.
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and Clustered Cell Surface Low Density Lipoprotein Receptor Molecules,"  
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**INVITED LECTURES, 2003-1992**  
*by Watt W. Webb except where noted*  
As of November 22, 2002

**2003**

- August 3-7, 2003: Title TBA, Microscopy & Microanalysis 2003, San Antonio, TX
- June 15-20, 2003: "Non-linear optical microscopy of the dynamics of molecular processes of living systems at single molecule sensitivity," Gordon Research Conference on Analytical Chemistry, Connecticut College, New London, CT
- April 28-29, 2003: Title TBA, Argonne National Laboratory, Argonne, IL
- January 26, 2003: "Correlation Spectroscopy Now," BIOS 2003, Multiphoton Microscopy in the Biomedical Sciences III, Photonics West 2003, San Jose, CA

**2002**

- October 15, 2002: "Multiphoton Imaging and Correlation Spectroscopy of the Molecular Dynamics of Life," Center for Analytical Biotechnology Lecture, UC-Berkeley, CA
- July 23, 2002: "A New Single Molecule Sequencing System," NIH National Human Genome Research Institute Meeting 'Sequencing and Resequencing the Biome,' Bethesda, MD
- July 29, 2002: Ahmed Heikal, "Multiphoton fluorescence microscopy for functional imaging of biomolecules," NLO, Nonlinear Optics: Materials, Fundamentals and Applications, Wailea, Maui, Hawaii
- July 7, 2002: Ahmed Heikal, "Multiphoton fluorescence microscopy in biology," SPIE Annual Meeting 2002, Seattle, WA
- June 25-29, 2002: "Sub-Optical Resolution Access to Enzymatic Kinetics," 5<sup>th</sup> International Weber Symposium, Kalapaki Beach, Lihue, Hawaii
- June 23-25, 2002: Warren Zipfel, "Nonlinear Microscopy and Nanotechnology: Tools for Systems Biology", NCRR P41 Principle Investigators Meeting, Washington, DC
- May 23, 2002: "Multiphoton Imaging the Molecular Dynamics of Life Processes," General Chemistry Colloquium, University of Washington
- May 22, 2002, Michael Levene, "Zero-mode waveguides for single molecule analysis and fast DNA sequencing," CLEO/QELS 2002, Long Beach, CA
- April 16-19, 2002: "Technological Challenges and Opportunities that Advance the State of the Art of 'Imaging' for Research on 'Genomes to Life'," DOE Genome Imaging Conference, Charlotte, NC

February 23-27, 2002: "Multiphoton Imaging the Molecular Dynamics of Living," National Lecture, Biophysical Society 46<sup>th</sup> Annual Meeting, San Francisco, CA

January 29, 2002: Warren Zipfel, "Optics, Electronics and Imaging - Getting Data from a Nanodevice," Nanobiotechnology Center Technology Platform Series, Cornell University, Ithaca, NY

January 27-31, 2002: Jonas Koriach, "Method for fast and highly parallel single molecule DNA sequencing," DOE Ninth Genome Contractor and Grantee Workshop, Oakland, CA

## **2001**

December 16-20, 2001: "Nanophotonics," Sixth International Conference on Organic Nonlinear Optics, ICONO'6, Tucson, AZ

November 1, 2001: "Observing the dynamical biophysical chemistry of life processes", Harvard/MIT Physical Chemistry Seminar, Harvard University, Cambridge, MA

October 11, 2001: "Multiphoton imaging of the molecular dynamics of life processes," New York Society for Microscopy symposium 'Frontiers of Microscopy,' Rockefeller University, New York

October 7-12, 2001: Dan Larson, "Multiphoton Spectroscopy in Zeptoliter Volumes Using Optical Enhancement," Federation of Analytical Chemistry and Spectroscopy Societies (FACSS) 2001, Detroit.

September 26-28, 2001: "New results at the single molecule level and new sub-resolution optics," 7<sup>th</sup> Annual International Workshop on 'Single Molecule Detection and Ultrasensitive Analysis in the Life Sciences,' at PicoQuant in the Science and Technology Park, Berlin Adlershof.

September 23-25, 2001: "Fluctuations: Atomic and Molecular - Nanoscopic and Microscopic," Horizons in Biophysics 2001, Karolinska Institute, Stockholm, Sweden.

September 21, 2001: "Fluorescence probing the dynamics of life processes," Max-Planck Institute for Medical Research, Heidelberg, Germany

August 31, 2001: Mike Levene "Fundamentals of multi-photon excitation for imaging and spectroscopy," and "Applications of multiphoton excitation and other methods for confined volume spectroscopy," Fluorescence Spectroscopy and Fluorescence Microscopy in Biosciences, Aalborg, Denmark

June 25, 2001: "The Biomedical Challenges to Neuronal Imaging," NIH-NCRR PI Meeting, Bethesda, MD

April 24, 2001: "Optronic Measurements of Biophysical Dynamics at the Cellular and Molecular Level," What Physicists Can Measure, What Biologists Would Like to Measure seminar series, Brown University, Providence, RI



April 10-11, 2001: Ahmed Heikal, "Fluorescence Spectroscopy, Dynamics and Imaging of Designed Two-Photon Fluorescent Markers in Triton X-100 micelles and RBL Cells" Opto Northeast Regional Meeting on: Optoelectronics, Photonics, and Imaging, Rochester, NY.

March 25-31, 2001: Ahmed Heikal, "Molecular spectroscopy and dynamics of selected biomolecular systems," European Science Foundation Ultrafast technology and advanced microscopy applications to intra-cellular and biomolecule dynamics school, Cargese, Corsica, France

March 6-8, 2001: Ahmed Heikal, "Two-Photon Fluorescence Imaging," Pittcon 2001 Conference, New Orleans, LA

January 20-24, 2001: "Multiphoton Microscopy: a Biomedical Research Instrument to Invade the Clinic," SPIE Photonics West, San Jose, CA

## 2000

- November 4-9, 2000: Peter Kloppenburg, Society for Neuroscience Annual Meeting, New Orleans, LA
- October 25-26, 2000: Sam Hess, "The Effects of Focal Volume Optics on Experimental Artifacts and Signal to Noise in Fluorescence Correlation Spectroscopy," Dan Larson, "Fluorescence Correlation Spectroscopy in Heterogeneous Samples," Zeiss Fluorescence Correlation Spectroscopy symposium/workshop, St. Louis, MO
- October 23, 2000: Warren Zipfel, "GFP Multiphoton Imaging and Correlation Fluorescence Spectroscopy," Cold Spring Harbor Laboratory Colloquium, Cold Spring Harbor, NY
- September 22-23, 2000: "Photonic analysis of biomolecular and cellular dynamics *in vitro* and *in vivo*" Biophotonics Center Workshop, Case Western Reserve University, Cleveland, OH
- September 20-21, 2000: "Multiphoton Microscopy and Correlation Spectroscopy as Biomedical Research Tools," Physics Dept., University of Toronto, Toronto, Ontario
- August 20-24, 2000: "Fluorescence Correlation Spectroscopy," Optical Society of America Conference "Photon Correlation and Scattering 2000," Whistler, British Columbia
- July 1, 2000: Ahmed Heikal, "Light-Controlled Intramolecular Dynamics in Ecliptic Green Fluorescent Protein (EcGFP)," 13th International Congress on Photobiology, San Francisco, CA
- June 26-28, 2000: "Optical Microscopy," Plenary Lecture, National Institutes of Health, Bethesda, MD.
- June 13, 2000: "Multiphoton Microscopy," Acceptance Lecture, Rank Prize in Opto-Electronics 2000, The Royal Society of Medicine, London, UK.
- June 8, 2000: "Photophysics of Green Fluorescent Protein Analyzed by FCS and TCSPC," Wenner-Gren Foundations Distinguished Lecture, Sweden, Göteborg University, Göteborg, Sweden.
- June 7, 2000: "Molecular Autofluorescence of Tissues Revealed by Multiphoton Microscopy," Wenner-Gren Foundations Distinguished Lecture, Sweden, Department of Physics, Lund University, Lund, Sweden
- June 5, 2000: "Workshop on Multiphoton Microscopy," Wenner-Gren Foundations Distinguished Lecture, Sweden, Nobel Forum, Stockholm, Sweden
- June 5: "Photophysics of Green Fluorescent Protein Mutants by FCS and TCSPC," Wenner-Gren Foundations Distinguished Lecture, Sweden, Karolinska Hospital, Stockholm, Sweden.
- April 12, 2000: "Biophysics with Multiphoton Microscopy and Correlation Spectroscopy Fluorescence," Physics Colloquium, University of Rochester, Rochester, NY.

March 14, 2000: "Fluorescence Correlation Spectroscopy probing biomolecular dynamics," Chemistry Seminar, Penn State University, State College, PA.

March 15, 2000: "Multiphoton Microscopy probing biological autofluorescence" Physics Seminar, Penn State University, State College, PA.

February 28, 2000: "Biomedical Research Applications of Multiphoton Microscopy," Biophysics Colloquium, Johns Hopkins University School of Medicine, Baltimore, MD.

February 27, 2000 Jonas Korlach, "New Optical Methods for Sequencing Individual Molecules of DNA," 8th DOE Human Genome Contractor Grantee Workshop, Santa Fe, NM.

February 12-16, 2000: "Biomedical Applications of Fluorescence," Jablonski Prize Lecture, Annual Biophysical Society Meeting, New Orleans, LA.

January 20, 2000: "Multiphoton laser microscopy and time resolved correlation spectroscopy," University of Arizona Optical Sciences Colloquium, Tucson, AZ.

## 1999

December 1, 1999: "Multiphoton Excitation (MPE) Of Organic Molecules In Biological Materials," Materials Research Society Fall Meeting, Boston, MA

November 18, 1999: "New Optical Methods for Sequencing DNA Molecules," Eastern Analytical Symposium, Summerset, NJ.

October 28, 1999: "Some Research Paths from Physical Science to Biological Science" Physics Department Colloquium, University of Illinois at Urbana-Champaign.

October 8, 1999: "Dynamics of Green Fluorescent Protein Revealed by Fluorescence Correlation Spectroscopy" Duke University Medical Center, Durham, NC.

September 16, 1999: "Multiphoton Microscopy: Imaging Spectra and Dynamics of Molecular Function Deep in Living Tissue," In- Vivo Optical Imaging workshop at the National Institute of Health, Bethesda, MD.

September 12, 1999: Petra Schwille: "Advantages of Two-Photon Excitation in Intracellular Fluorescence Correlation Spectroscopy" 6<sup>th</sup> International Conference on Methods and Applications of Fluorescence Spectroscopy, Paris, France.

September 9, 1999: "Through the looking glass into the molecular dynamics of life," New Optical Methods in Cell Physiology, Woods Hole, Massachusetts. (Keynote Speaker)

September 1999: Jonas Korlach: "Advanced Optical Techniques for Biochemical Analysis" Optical Society of America 1999 National Meeting, Santa Clara, CA.

July 31, 1999: "Something New in Microscopy" Microscopy Society of America Pre-congressional meeting, Portland, Oregon.

- June 24, 1999: "Multiphoton Fluorescence Correlation Spectroscopy with Single Molecules in Living Cells," 4th International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine, Maui, Hawaii.
- July 1999: Michael Nichols: "Photodynamic Damage to Multicell Tumor Spheroids Observed by Two-Photon Microscopy of Sensitized and Endogenous Cell Fluorescence," 27<sup>th</sup> annual meeting of the American Society for Photobiology, Washington, DC.
- June 9, 1999: "Single Molecule Dynamics as Contextual Probes," Spectroscopy of Single Molecules in Physics, Chemistry and Life Sciences, Södergarn Mansion, Lidingö.
- May 4, 1999: "Biomedical Targeting of Microscopic Ultrafast Optics," Case Western Reserve, Cleveland, Ohio. (Michelson-Morley Prize Lecture)
- March 19, 1999: Warren Zipfel: "Multiphoton laser scanning fluorescence microscopy: the technique and its application", Oxford University, Oxford England. Invited by Nick White, Oxford University, Oxford, England.
- March 15, 1999: Warren Zipfel: "Multiphoton laser scanning fluorescence microscopy: the technique and its application", German Cell Biology Meeting, Rostock, Germany. Invited by Andrew Dixon, BioRad Laboratories, UK.
- February 26, 1999: "Multiphoton Microscopy" Nippon Bio-Rad Laboratories, Japan.
- February 25, 1999: "Conference Closing Summary" The 7<sup>th</sup> JST International Symposium Molecular Processes and Biosystems, Tokyo, Japan.
- February 25, 1999: "Cell Signaling Dynamics: of Molecular Signaling in Vivo and in Vitro" The 7<sup>th</sup> JST International Symposium Molecular Processes and Biosystems, Tokyo, Japan.
- February 8, 1999: "Observing Molecular Signaling Dynamics and Supramolecular Structures in Neuroscience," Mayo Clinic, Jacksonville, Florida.
- February 4, 1999: "Biological Physics with Ultrafast Multiphoton Microscopy and Correlation Spectroscopy" University of Florida, Gainesville, Florida.

## 1998

- December 11, 1998: "Biomedical Applications of Multiphoton Microscopy" Harvard University, Boston, MA.
- November 12, 1998: "Multiphoton Molecular Excitation and Fluorescence Correlation Spectroscopy Probe the Dynamics of Biological Processes," The Scripps Research Institute, La Jolla, CA.
- October 15, 1998: Warren Zipfel: "Multiphoton fluorescence microscopy of cells and tissues." 25th annual conference of the Federation of Analytical Chemistry and Spectroscopy Societies, Austin, TX. Invited by Jason Shear, University of Texas, Austin.

- October 9, 1998: Michael Nichols: "Simultaneous Two-Photon Imaging of Photofrin and NADH Autofluorescence in Cell Monolayers and Multicell Tumor Spheroids" Pharmacology Seminar Series at the University of Wisconsin-Madison.
- September 9, 1998: "Multiphoton Molecular Excitation Images Biological Functions," New Technology in Cell Biology and Genomics workshop, Howard Hughes Medical Institute, Chevy Chase, MD.
- August 30-Sept. 1, 1998: Petra Schwille: "Fluorescence correlation spectroscopy in the cellular environment employing nonlinear techniques," Symposium of Dynamics of Biological Process at the University of Bielefeld, Germany
- August 7-9, 1998: "Imaging Structure and Functions in the Nervous System" Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
- July 11, 1998: Warren Zipfel: Multiphoton Imaging in Highly Scattering Samples," Multiphoton Microscopy Satellite Meeting, Annual Meeting of the Microscopy Society of America, Atlanta, GA.
- June 2, 1998: Warren Zipfel: "Application of Multiphoton Microscopy in Neuroscience," UCLA Brain Research Institute, University of California at Los Angeles.
- May 30, 1998: Petra Schwille: "Fluorescence Correlation Spectroscopy (FCS): Ultrasensitive measurements of Molecular Dynamics in Vitro and in Vivo." American Physical Society, Santa Fe, CA
- April 26, 1998: Michael Nichols: "Biophysical Imaging with Multiphoton Microscopy," 46<sup>th</sup> Annual Meeting of the Radiation Research Society Conference, Louisville, KY.
- April 25, 1997: Warren Zipfel: "Multiphoton Microscopy Training Course," Bio-Rad, Hercules, CA.
- February 28, 1998: "Multiphoton Microscopy: Fundamental principles, advantages and disadvantages" Bio-Rad, San Francisco, CA
- February 26, 1998: "Precision Fluorescence Imaging of a Single Molecule vis-a-vis the Abbe Criterion," Ernst Abbe Lecture, Symposium on Single Molecules at Work, Biophysical Society Annual Meeting, Kansas City, MO.
- February 13, 1998: "Multiphoton Excitation as a Microscopic Probe of Biological Function," Biochemistry Molecular and Cell Biology Colloquium, Cornell University, Ithaca, NY.
- February 10, 1998: Dr. Sudipta Maiti: "Protonation Fluctuations Make GFP Flicker," Biophysics Colloquium, Cornell University, Ithaca, NY.
- January 12, 1998: "Dynamics of Individual Biomolecules," Japan/US Exchange Seminar on Photophysics and Photoconversion in Small Domains, Napa, CA.
- January 8, 1998: " Multiphoton Molecular Excitation," Theoretical and Physics Colloquium, Los Alamos National Laboratory, Los Alamos, NM.

January 7, 1998: "Time dependence of Fluorescence form Green Fluorescent Protein," Flow Cytometry Seminar Los Alamos National Laboratory, Los Alamos, NM.

## 1997

December 10, 1997: "Biomedical Application of Multiphoton Laser Microscopy," Molecular Biophysics Seminar - Washington University School of Medicine, St. Louis, MO.

December 9, 1997: "A Biophysical Evening of Infrared into Ultraviolet," Molecular Biophysics Seminar- Washington University School of Medicine, St. Louis, MO.

December 4, 1997: "Biomedical Applications of Multiphoton Microscopy," Physics Colloquium, Northeastern University, Boston, MA.

November 13, 1997: Warren Zipfel: "Multiphoton excitation imaging and photochemistry in cells and tissue," Advances in Cellular Imaging for Biological Research and Drug Development, San Diego, CA.

November 12, 1997: "Multiphoton Microscopy Probes the Molecular Processes of Living Cells," Spectroscopy Societies of Pittsburgh, Pittsburgh, PA.

November 11, 1997: Warren Zipfel: "Application of Multiphoton Microscopy," Martin Fridlander Laboratory, Scripps Institute, La Jolla, CA.

November 3, 1997: "Biomedical Applications of Multiphoton Microscopy," SUNY Buffalo Medical College, Buffalo, NY.

October 29, 1997: "Imaging Secretion of Serotonin and Related Indolamines with Multiphoton Microscopy Symposium on Optical Imaging of Presynaptic Function," Society of Neuroscience Annual Meeting, New Orleans, LA.

October 21, 1997: "Biomedical Applicators of Multiphoton Microscopy, Physics Colloquium, Rockefeller University, New York City, NY.

October 3, 1997: "Applications of Multiphoton excitation imaging in the Plant Sciences," SR Noble Foundation, Ardmore, OK

August 11, 1997: "Biological Applications of Multi-Photon Excitation Fluorescence Imaging," Microscopy and Microanalysis '97, Cleveland, OH.

August 10, 1997: Rebecca Williams: "Three-photon excited fluorescence microscopy of serotonin release," Applications of Multiple Photon Excitation Imaging Symposium and Short Course, Cleveland, OH.

August 9, 1997: Warren Zipfel: "Multi-photon excitation of intrinsic fluorescence in cells and intact tissue," Applications of Multiple Photon Excitation Imaging Symposium and Short Course, Cleveland, OH.

May 23, 1997: "Biophysical Imaging," (actually presented by Dr. Mike Nichols) Quantum Electronics and Laser Sciences Conference, Baltimore, MD.

- April 15, 1997: "Single Molecule Trajectories Reflecting Non-Linear Bimolecular and Photophysical Dynamics in Cells and Solutions," Symposium on Chemistry of Single Molecules at American Chemical Society Annual Meeting, San Francisco, CA.
- March 26, 1997: "Biomedical Applications of Non-Linear Laser Microscopy," Department of Biology Colloquium, Yale University, New Haven, CT.
- March 25, 1997: "Biophysical Dynamics Illuminated by Non-Linear Laser Microscopy," Lucent Technologies - Bell Laboratories, Murray Hill, NJ.
- February 25, 1997: "Three Dimensional Optical Data Storage," Eastman Kodak, Rochester, NY.
- February 24, 1997: "Biological Applications of Non-Linear Laser Microscopy," Harvard University Colloquium, Cambridge, MA.
- February 12, 1997: "Two-Photon Imaging of Skin," Conference on Functional Imaging and Optical Manipulation of Living Cells, SPIE-BIOS'97, San Jose, CA.
- January 31, 1997: "Non-Linear Laser Microscopy," Quantum Optics in Biology and Medicine, CIBA Foundation/Royal Society Discussion Meeting, London, England.
- January 27, 1997: "Biological Applications of Non-Linear Laser Microscopy," Advanced Solid State Lasers, Orlando, Florida.

## 1996

- October 9, 1996: "Nonlinear Laser Microscopy Illuminates Biomedical Dynamics," Lund Technical University Medical Physics Colloquium, Lund, Sweden.
- October 8, 1996: "Non-Linear Laser Microscopy," Karolinska Institut, Stockholm, Sweden.
- October 7, 1996: "Biomedical Applications of Non-Linear Laser Microscopy," Karolinska Institut Medical Physics Seminar, Stockholm, Sweden.
- September 28, 1996, "Biological Applications of Non-Linear Laser Microscopes," Institute Curie, Paris, France.
- September 25, 1996: "Non-Linear Laser Microscopy," Max Planck Institute, Goettingen, Germany.
- September 25, 1996: "Technical Seminar Neher Laboratory," Max Planck Institute Goettingen, Germany.
- September 24, 1996: "Dynamical Cage Activation Micropharmacology by Multiphoton Excitation," Caged Compounds Conference, Schloss Reisenberg, Germany.
- August 6, 1996: "Principles of Two-Photon Microscopy," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- June 17, 1996: "Non-Linear Laser Microscopy," American Society for Photobiology, Atlanta, GA.

June 4, 1996: "Two-Photon Excited Confocal Microscopy," CLEO/QELS, Anaheim, CA.  
 May 29, 1996: "Multi-Photon Molecular Excitation to Illuminate Non-Linear Laser Microscopy," Chris Xu and Watt W. Webb, Ultrafast Phenomena, San Diego, CA.  
 April 14, 1996: "Current Biophysical Research Topics," CIA Workshop, Washington, DC.  
 April 11, 1996: "Analytical Non-Linear Microscopy in Living Tissues and Cells," Schepens Eye Research Institute, Boston, MA.  
 February 21, 1996: "Non-Linear Optical Microscopy," Biophysical Society Meeting, Baltimore, MD.  
 February 9, 1996: "Non-Linear Laser Microscopy," AAAS meeting, Baltimore, MD.

### 1995

October 27, 1995: "Non-Linear Excitation and Optical Probes of Microchemistry and Surface Dynamics in Biophysics," Columbia University, New York, NY.  
 September 30, 1995: "What Molecular Motion Trajectories Tell us About Nanometer Cell Surface Domains?" Australia and New Zealand Society for Cell Biology, International Conference, Canberra, Australia.  
 September 26, 1995: "Physics and Biophysics," University of Sydney, Australia.  
 September 15, 1995: "Two-Photon Excitation in Laser Scanning Microscopy," Optical Society of America, Portland, OR.  
 September 15, 1995: "Two-Photon and Near-Field Microscopy," National Research Council, Portland, OR.  
 August 17, 1995: "What Do Nanometer Molecular Trajectories Tell Us About Heterogeneous Cell Surface Domains?" Membranes and Microdomains Symposium, Annual Meeting, Microscopy Society of America, Kansas City, KS.  
 August 8, 1995: "Two-Photon Microscopy Principles," Imaging Structure & Function in the Nervous System, Cold Spring Harbor, NY.  
 June 14, 1995: "Laser Biophysics," Twelveth International Conference on Laser Spectroscopy '95, Capri, Italy.  
 May 2, 1995: "Can Two-Photon Excitation Illuminate Medical Applications of Laser Microscopy?" Optical Probes in Biology and Medicine Workshop, Cambridge, MA.  
 April 20, 1995: "Recent Developments in Non-Linear Microscopy Illuminated by Two-Photon Excitation," Focus on Microscopy '95 Conference, Taipei, Taiwan.  
 February 25, 1995: "What Do Nanometer Molecular Trajectories Tell Us About Nanometer Heterogeneities in Cell Surfaces?" Nanobiology Workshop, Keio University, Tokyo, Japan.



February 23, 1995: "Neuroscience Research Illuminated by Two-Photon Excitation in Non-Linear Laser Microscopy", Frontier Research Programs, Riken, Japan.

February 10, 1995: "Physiological Application of Two-Photon Excitation in Non-Linear Laser Microscopy," NIH Workshop on Optical Techniques for the Study of Physiological Processes; Recent Advances and Future Directions, Napa, CA.

## **1994**

October 17, 1994: "New Developments in Two-Photon Excitation Laser Microscopy," XVII Meeting of the International Society for Analytical Cytology, Lake Placid, NY.

October 2-4, 1994: "Optical Force Microscopy," Lucien Ghislain and Watt Webb, Optical Society of America Annual Meeting, Dallas, TX.

September 19, 1994: "Physical Optics Empowers Microscopic Manipulations and Imaging of Dynamics of Cellular Biophysics," Washington University, St. Louis, MO.

August 10, 1994: "Two-Photon Microscopy," Imaging Structure and Function in the Nervous System, Cold Spring Harbor, NY.

June 13, 1994: "Physical Optics Empowers Microscopic Manipulations and Visualization of Dynamic Cellular Mechanisms," International Conference on Contributions of Biomedical Engineering to Biology and Medicine, Bethesda, MD.

May 13, 1994: "Cornell Technologies," Cornell Technology Transfer Committee: Venture Capital Conference, New York, NY.

April 26, 1994: "Optical Force Microscopy," International Conference on Confocal and Near-Field Microscopy, Munich, Germany.

March 11, 1994: "Two-Photon Excitation to Illuminate Biophysics," Keck Symposium on Biophysical Applications of Microscopy, Mayo Foundation, Rochester, MA.

March 7, 1994: "Fractal TimeTransport in the Cell Surface," Biophysical Society Annual Meeting, Symposium on Surface Particle Movements and Membrane Dynamics, New Orleans, LA.

March 2-4, 1994: "Visualization and Measurement of Membrane Domains," NIH Fogarty International Center Conference on Domains Organization in Biological Membranes, Bethesda, MD.

January 13-14, 1994: "Advanced Technologies in Neuroscience," National Institute of Mental Health, Rockville, MD.

## **1993**

November 18, 1993: "Three-Dimensional Imaging with Two-Photon Fluorescence," Alliance for Photonic Technology, Albuquerque, NM. (videotape available).

August 6-8, 1993: "Two-Photon Excitation in Laser Scanning Microscopy Fluorescence Photobleaching Recovery," Cold Spring Harbor Laboratories; summer course on Imaging in Neurobiology, Cold Spring Harbor, NY.

August 1, 1993: "Two-Photon Excitation Illuminates Cage Photolysis and Molecular Fluorescence for Visualization and Measurement of Dynamic Cellular Processes," Conference on Two Photon Photochemistry, Boston, MA. (Presented by David Sandison)

May 19-20, 1993: Microscopy Course - Plenary Lecture on "Two Photon-Excitation in Laser Scanning Microscopes," Marine Biological Laboratories, Woods Hole, MA.

May 7, 1993: "Visualizing the Physics of Life in the Cell," 1993 Bertman Memorial Lecture, Wesleyan University, Middletown, CT. (videotape available)

February 20, 1993: "Two-Photon Excitation Visualizes Dynamics of Molecular Processes Inside the Living Cell," University of Illinois - Nalbandov Memorial Symposium on Inside the Living Cell, Urbana, IL.

January 29, 1993: "Biological Applications of Non-Linear Two-Photon Laser Microscopy," Inaugural Symposium of the Microscopy Committee - Neurobiology and Behavior, and Biophysics, SUNY-Stony Brook, Stony Brook, NY.

## 1992

December 7, 1992: "Non-Linear Microscopies," Symposium of Center for Light Microscope Imaging and Biotechnology, Carnegie Mellon University, Pittsburgh, PA.

September 24-25, 1992: "Emerging Technologies Illuminates Cellular Dynamics: Non-Linear Microscopes," NIH Workshop Technologies for the Future, Bethesda, MD. (Also chair of discussion group in instrumentation hardware.)

September 4, 1992: David Sandison substituting for W.W. Webb, "Two-Photon Imaging in Biological Microscopy," 9th International Congress of Histochemistry and Cytochemistry, Maastricht, The Netherlands.

July 21-25, 1992: "Non-Linear Laser Microscopy," AAAS Science Innovation '92, San Francisco, CA.

May 21, 1992: "Membrane Surface Dynamics," Conference on Fundamental Concepts in Membrane Biophysics, McMaster University, Hamilton, Ontario, Canada.

April 23, 1992: "Subcellular Photo-Chemical Microsurgery Innovation: From Concept to Market Place," 9th Annual Engineering Conference, Cornell University, Ithaca, NY.

April 10-11, 1992: "Two-Photon Excitation in Laser Microscopy and Molecular Dynamics in Cellular Biophysics," American Physical Society, New York State Section, Syracuse, NY.

April 3, 1992: "Biophysics at Cornell," Cornell Engineering College Council, Ithaca, NY.

March 9, 1992: "Non-Linear Laser Scanning Microscopy: Two-Photon Excitation Provides 4-D Resolution Fluorescence and Photochemistry," Opening Lecture, 4th International Conference on Confocal Microscopy, Amsterdam, The Netherlands.

February 11, 1992: "Membrane Dynamics in the Light Microscope," AAAS Annual Meeting, Symposium on the Revolution in Microscopy, Chicago, IL.